A 1998 Survey of Extended-Spectrum β-Lactamases in Enterobacteriaceae in France

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In a 3-month period in 1998, 79 consecutive isolates of Enterobacteriaceae producing an extended-spectrum β-lactamase (ESBL) were collected. ESBLs were predominantly TEM derivatives (74 of 79): TEM-24-like (40 isolates), TEM-3-like (29 isolates), TEM-21 (3 isolates), and TEM-4 and TEM-52 (1 isolate each). Four isolates produced SHV derivatives SHV-4 (three isolates) and SHV-5 (one isolate), and one strain produced a CTX-M-3 enzyme. The high proportion of TEM-24-like-producing Enterobacter aerogenes isolates (36 of 79) suggests the occurrence of an epidemic strain in France.

Extended-spectrum β-lactamases (ESBL) are now observed in all species of Enterobacteriaceae. Many of these enzymes are TEM or SHV derivatives and other newly emerging class A enzymes such as PER-1 and CTX-M. To evaluate the frequency and the diversity of ESBL produced by strains of Enterobacteriaceae in France, a survey was conducted from April to June 1998 in 14 French hospitals, 7 of 29 metropolitan university hospitals, and 7 nonteaching hospitals in 11 different regions including Ile-de-France, the Paris region (Fig. 1). The first 180 consecutive nonduplicate isolates of each laboratory were screened for ESBL by the double-disc synergy test. They were identified using automated systems. In all laboratories, antibiotic susceptibility testing was performed on Mueller-Hinton agar and with antibiotic discs from Diagnostic Pasteur (Marnes la Coquette, France), placed at defined positions. Strains were collected if the synergy test was positive. Isolates from superficial wounds, stools, ear, nose, and throat, those not involved in infections according to the Centers for Disease Control and Prevention criteria (8), and those from swab samples were excluded. Once weekly, Escherichia coli ATCC 25922 and E. coli C600/pCFF04 (TEM-3) were tested as quality controls.

The β-lactamases were characterized by isoelectric focusing. blaTEM genes were then detected by PCR using primers A (5′TAA-AAT-TCT-TGA-AGA-CG-3′) and B (5′TTA-CCA-ATG-CTT-AAT-CA-3′), and known mutations at positions 39, 104, 164, and 238 were detected by allele-specific PCR (ASPCR) (16). blaSHV genes were detected with primers OS5 (5′TTA-TCT-CCC-TGT-TAG-CCA-3′) and OS6 (5′GAT-TT G-CTG-ATT-TCG-CTC-3′). The ESBL that was neither a TEM nor a SHV derivative was identified by direct sequencing of the PCR product obtained with specific primers chosen according to the isoelectric point value (8.4) and the resistance phenotype (high-level resistance to cefotaxime), both of which point to a CTX-M-3 enzyme (9). For each ESBL TEM type in each species and each region (n = 23), direct DNA sequencing of PCR products was performed to confirm the mutations observed by ASPCR or to detect any mutation hitherto unseen. For the SHV genes, sequences were obtained for one SHV-4 gene and one SHV-5 gene after plasmid transfer in E. coli. To study the clonal diversity of the strains, ribotyping was performed for the 36 TEM-24-like ESBL- and 7 TEM-3-like ESBL-producing Enterobacter aerogenes isolates and 11 TEM-3-like ESBL-producing Klebsiella pneumoniae isolates with a cold-labeled probe obtained from E. coli ribosomal 16S and 23S RNA (Boehringer GmbH, Mannheim, Germany).

Of the 2,506 Enterobacteriaceae isolated, 79 strains produced ESBL, a higher proportion (3.2%) than in 1990 (1.5%) and 1991 (0.9%) (10, 15). For E. coli species, this proportion did not increase from 1990 (1.5%) to 1991 (0.9%) to 1996 (0.1%) to 1998 (0.2%). The increase in the proportion of ESBL-producing Proteus mirabilis strains (0% in 1990 versus 3.7% in 1998) may have been underestimated. Frequencies close to 6% were observed in Clermont-Ferrand from 1996 to 1998 (6, 7). In P. mirabilis the synergy between extended-spectrum cephalosporins and clavulanate can be difficult to detect by disk diffusion. For K. pneumoniae the percentage in 1998 (9.4%) did not differ significantly from that in 1991 (9.6%). The increases observed in 1994 (22.2%) and in 1996 (17.7%) were apparently transient (6; M. H. Nicholas-Chanoine, H. Chardon, and the Multicenter Group, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2253, 1999). The proportion of ESBL-producing E. aerogenes isolates rose from <2.5% in 1990 to 53.5% in 1998 (15). This high occurrence may also have been transient and due to an epidemic strain, as reported in a survey in which the prevalence decreased from 20.2% in 1990 to 5.2% in 1994 (6). Without the 43 epidemic E. aerogenes isolates, the ESBL rate was 1.5%. The proportion of ESBL-producing strains among Enterobacteriaceae varied from 1.6% in hematology units to 7.1% in intensive care units to 7.7% in rehabilitation units. The proportions of Enterobacteriaceae isolated from the different samples were as follows: 4 of 29 (13.8%) from extravascular catheters, 4 of 38 (10.5%) from stools, 10 of 187 (5.3%) from the respiratory tract, 49 of 1,681 (2.9%) from urine, 5 of 219 (2.3%) from blood cultures, and 7 of 352 (2.0%) from wounds and serous effusions.

Eight different ESBL were characterized: TEM-24-like (40 isolates, 50.62%), TEM-3-like (29 isolates, 36.70%), TEM-21 and SHV-4 (3 isolates each, 3.80%), and TEM-4, TEM-52, SHV-5, and CTX-M-3 (1 isolate each, 1.27%) (Table 1). Their

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geographical distribution is shown in Fig. 1. In three centers, Rennes, Le Mans, and Troyes, no ESBL were detected. TEM-24-like ESBL- and TEM-3-like ESBL-producing strains were observed in seven and eight centers, respectively. The TEM-24-like ESBL was the most frequent in *E. aerogenes* isolates (36 of 46; 78.3%). The TEM-24-like ESBL- and TEM-3-like ESBL-producing *E. aerogenes* isolates revealed only two different ribotypes according to their ESBL. This is suggestive of the spread of the two clonal strains. TEM-24-like ESBL-producing strain outbreaks were reported elsewhere (4, 13), and all TEM-3-like ESBL-producing *E. aerogenes* strains were isolated in the same region. TEM-3-like enzyme was observed in 11 of 13 (84.6%) *K. pneumoniae* isolates belonging to five different ribotypes.

When ESBL appeared, they were predominantly TEM-type enzymes (10), but in Europe since the mid-1990s the SHV-type ESBL is the most frequent (1). The greater incidence of the SHV-type ESBL seems related to the predominance of *K. pneumoniae* among the ESBL-producing strains (5, 6, 7, 11). In the present survey, the TEM-type ESBL was again the most frequent and concomitantly *E. aerogenes* was the most frequent of the ESBL-producing *Enterobacteriaceae*. This may be because TEM-24 is one of the most efficient ESBL owing to the combination of four amino acid substitutions at positions 104, 164, 237, 240, which are known to enhance the hydrolysis of oxyimino-amino thiazole cephalosporins (12). In addition the presence of T at position 32 in the \( \text{bla}_{\text{TEM-24}} \) gene promoter leads to an increase in transcriptional level. Further studies are needed to understand why TEM-24-producing *E. aerogenes* is currently the predominant ESBL-producing species of the *Enterobacteriaceae* in France. In this survey a CTX-M enzyme (CTX-M-3) was observed in *Enterobacter cloacae* in a patient residing in France and admitted to Versailles hospital. CTX-M-3 was reported in Poland in 1996 (9). The class A cefotaxime-hydrtylizing enzymes (CTX-M) do not derive from TEM and SHV. Most of these enzymes have been observed in South America, Israel, Japan, and eastern Europe (3).

The differences in the frequencies of ESBL-producing strains between regions and from year to year are in part due to epidemic strains. In Europe an increasing proportion of ESBL-producing strains among *Enterobacteriaceae* has been reported (1). New mutant TEM or SHV and non-TEM, non-SHV class A enzymes are still being reported and are spreading worldwide to all species of *Enterobacteriaceae* and other genera such as *Pseudomonas* (2, 3, 11, 14). This underlines the need for regular surveillance of the prevalence of these enzymes and for continuing vigilance in prevention.

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**APPENDIX**

Participating members of the French Study Group: J. L. Avril (Rennes), C. Cattoen (Valenciennes), H. Chardon (Aix-en-Provence), J. L. Croix (Troyes), H. Dabernet (Toulouse), T. Fosse (Nîce), J. C. Ghnassia (Versailles), E. Lecaillon (Perpignan), A. Marmonier (Le Mans), M. H. Nicolas-Chanoine (Boulogne), M. Roussel-Delvallez (Lille), J. Sirot (Clermont-Ferrand), C. J. Soussy (Créteil), A. Trevoux (Mullhouse), F. Vandenesch (Lyon).

**TABLE 1. Distribution of ESBL according to species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total no. of isolates</th>
<th>Total (%)</th>
<th>TEM-24-like</th>
<th>TEM-3-like</th>
<th>Others (ESBL type)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>1,606</td>
<td>4 (0.2)</td>
<td>3</td>
<td>0</td>
<td>(TEM-52)</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>215</td>
<td>8 (3.7)</td>
<td>0</td>
<td>5</td>
<td>3 (TEM-21)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>138</td>
<td>13 (9.4)</td>
<td>1</td>
<td>11</td>
<td>1 (SHV-5)</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>86</td>
<td>46 (53.5)</td>
<td>36</td>
<td>7</td>
<td>3 (SHV-4-like)</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>32</td>
<td>1 (3.1)</td>
<td>0</td>
<td>0</td>
<td>1 (TEM-4)</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>30</td>
<td>2 (6.7)</td>
<td>0</td>
<td>1</td>
<td>1 (CTX-M-3)</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>30</td>
<td>5 (16.7)</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Others*</td>
<td>369</td>
<td>0 (0.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Total            | 2,506                 | 79 (3.2)  | 40          | 29         | 10                 |

*Citrobacter freundii, Enterobacter spp., Hafnia alvei, Klebsiella oxytoca, Proteus spp., Providencia spp., Yersinia enterocolitica.*

**REFERENCES**